1. Scientific Abstract

Laminin 5 beta 3 Gene Therapy for Junctional Epidermolysis Bullosa

Epidermolysis bullosa (EB) is a family of genetically inherited blistering skin disorders. Children born lacking the protein laminin 5 β3 develop the most severe subtype of EB, Herlitz junctional epidermolysis bullosa (JEB), and are plagued by painful blisters and ulcerations on their skin and mucosa. Ninety percent of affected children die within the first year of life, usually of sepsis or pulmonary failure (National Epidermolysis Bullosa Registry Data). The current therapy for EB consists of palliative wound care and there are currently no therapies available that alter the course or severity of the disease.

We have been able to demonstrate in an animal model that JEB patient keratinocytes can be grown in culture, transduced with a retroviral expression vector capable of sustained cutaneous gene expression for laminin 5 β, and grafted onto immune-deficient mice. We plan to use the same approach to generate corrected autologous keratinocyte sheets and graft them onto blistered, ulcerated areas of diseased skin of JEB patients. In an open label study of 10 patients, we plan to evaluate whether 2 transduced keratinocyte grafts, no greater than 50 cm^2 in size each, can attach to affected patients' skin and express laminin 5 at the basement membrane zone, as determined by clinical exam and immunofluoresence, 6 weeks and 6 months after grafting. These graft sites will be compared to two ungrafted sites, which will be treated with conventional wound care. The patients will be followed at least twice a year after the six month treatment trial is completed as part of a follow-up protocol. The initial clinical protocol, which has subsequently been revised, was approved by Stanford University Human Subjects Review Board on 4/4/2000. We also received approval from the Administrative Panel on Biosafety at Stanford on 4/10/2000.

In the proposed study, patient keratinocytes will be obtained from consenting and eligible patients and/or their proxies and transported to Genzyme Corporation, Framingham, Massachusetts where keratinocytes will be grown using techniques employed in the production of their autologous keratinocyte graft product Epicel. At Genzyme, the child's keratinocytes will then be transduced by insertion of the laminin 5 b3 corrective gene using the retroviral expression vector developed at Stanford and manufactured by the National Gene Vector Laboratories (NGVL-IU), Indianapolis. (Approval for manufacture was obtained 9/26/00; Master File BBMS8653). Good Manufacturing Practices (GMP), appropriate to the Phase I Clinical trial will be used in all steps of this manufacturing process. We will be submitting our application to the Recombinant Advisory Committee of the NIH at the next deadline, October 18, 2000.

Background on Vector and me-clinical Experiments

To date, retroviral vectors have been developed that sustain gene transfer and restore normal gene expression and adhesion to both JEB cells and regenerated skin tissue in vivo. Our study will utilize a recombinant, non-replication competent retroviral vector for the laminin 5 β3 gene. No helper virus or wild-type virus will be used at any time. This Moloney murine leukemia virus (MLV)-based retroviral vector was constructed for high efficiency ex-vivo gene transfer to JEB autologous patient keratinocytes prior to autologous grafting of such genetically engineered cells back to the patient. It is a self-inactivating vector containing a deletion of sequences in the U3 region of the 3' long terminal repeat (LTR) that has proven capable of providing gene delivery sustainable through multiple human epidermal turnover cycles in human skin regenerated on SCID mice. (Deng et al., 1997) The deleted vector sequences were previously shown to be involved in inactivation of the retroviral LTR in tissue over time and were not replaced with other sequences. When used at $\geq 5x10^6$ infectious particles per ml, these vectors can deliver genes to >95% of target primary keratinocytes. Since gene transfer is ex vivo, there is no spread of delivered gene expression beyond epidermal keratinocytes.

In the case of JEB, our corrective laminin 5 β3 gene delivery has been sustainable for multiple epidermal turnover cycles out to 8 weeks post-grafting, the duration of the experiment. Based on studies which have demonstrated that if retroviral vectors are able to sustain gene expression past the 3 to 4 week epidermal turnover period, they can last at least 40 weeks in vivo (Kolodka et a1.,1998), gene expression may in this scenario last indefinitely. We have confirmed in other settings that these vectors sustain gene expression for 3 months in other diseases through multiple epidermal turnover cycles without adverse cutaneous or systemic effects (Deng et al., 1997).

In the course of confirming the corrective and sustainable gene delivery in the treatment of a SCID mouse model of JEB using the IN-β3 vector, toxicity has not been observed. Specifically, the following have been demonstrated:

1. There was no increased mortality in over 100 immune deficient mice (SCID mouse model) grafted with genetically engineered human skin tissue using the therapeutic or marker vectors described below compared with non-engineered tissue controls. Retroviral vectors used for ex vivo gene transfer in these and related studies include those expressing the GFP and LacZ marker genes, as well as human TGase1, Arylsulfatase C, laminin 5 beta 3, Type XVII collagen genes.

2. There has been no evidence of delivered gene expression beyond the genetically engineered graft. This finding is not surprising since all exposure to the non-replication-competent retroviral vectors occurs in vitro prior to grafting.

3. No abnormalities or neoplastic changes have been demonstrated in any internal organ on gross and microscopic necropsy analyses in any laminin 5 beta 3-treated SCID mice, with the exception of the reactive myeloid changes that are seen with all mice that receive skin grafts, including those receiving non-engineered control human grafts. There was no evidence of neoplasia in laminin 5 beta 3 treated grafts up to 8 weeks old. One mouse, treated with a transduced GFP marker gene graft for 3 weeks, was noted on autopsy to have a malignant schwannoma. It is probable that this malignancy developed prior to grafting.

4. There is no evidence that the retroviral vector recombined with any endogenous viral sequences in the animals. Furthermore, this vector is a standard MLV-based vector with the slight modification that it is driven by internal promoter and has a partial deletion of the retroviral LTR. This deletion should be slightly safer than earlier generation constructs because there is no intact LTR to support the viral life cycle.

5.Our vector is replication-deficient and, to our knowledge, none of these cells will produce infectious particles. The 293T -based Phoenix packaging line has been used to produce these retrovirus preparations.

6. We do not have systematic information on rearrangement, recombination, or mutation. Because we have observed stable gene expression through multiple epidermal turnover cycles, we predict that the proviral genome is stable and the expression is stable. The half-life of laminin 5 protein in vivo appears to be less than a week from studies based on protein delivery experiments. Thus, we believe that the proviral genome is intact and will continue to deliver laminin 5 protein for long periods.

Risks and Benefits

Individual patients in this trial may be made slightly more comfortable by the grafting, but are likely to derive little benefit from this initial study. If, however, this approach proves feasible, substantial benefit could be derived by these and other patients in the future.

The major risks of concern include:

1. Immunological reactions similar to the immunobullous diseases such as bullous pemphigoid or acquired epidermolysis bullosa. In such a case, patients may need to be treated with immunosuppressive drugs and/or have the graft removed.

2. Risk of malignant transformation of the cells transplanted to the patient. Tumors or transformation have not been observed in the grafts in the animal model, but since the patients may maintain their grafts for extended periods of time, they will need to be monitored carefully.

3. Laminin 5 β3 deficient cells that are corrected with gene therapy have increased adhesion and mobility. These grafts may displace the unblistered genetically laminin 5 β3 deficient normal skin of these children. We will be monitoring to evaluate whether the grafts expand and plan to select grafting sites well away from mucous membranes in this protocol.

4. The patients will be monitored at least annually for the development of replication competent virus.

5. Since the procedure involves ex vivo gene transfer under controlled laboratory conditions using non-replication competent retrovirus, it is extremely unlikely that health care workers, the patients' immediate families, or other individuals would be at risk for viral shedding. Previous studies using non-replication competent retrovirus have not demonstrated any significant possibility that the added DNA will spread from the patient to other persons or to the environment. Standard retroviral precautions will be used throughout the protocol.

This study will likely be the earliest clinical trial using gene therapy for cutaneous skin diseases. Although there is substantial risk, we propose that its use is justified in these high risk patients who suffer from a severe life-threatening condition for which there are currently no other therapeutic alternatives that can change the severity or course of the disease.